IN THE UNITED STATES

PATENT AND TRADEMARK OFFICE

APPLICANTS:	Singh et al.
APPLICATION NO.:	10/740,079
FILING DATE:	December 18, 2003
TITLE:	Cell Screening Assay And Composition
EXAMINER:	Walter Schlapkohl
GROUP ART UNIT:	1636
ATTY. DKT. NO.:	25237-12174 US/089.00US

DECLARATION OF AHMED CHENNA, PH.D. UNDER 37 C.F.R. § 1.132

Sir:

I, Ahmed Chenna, Ph.D., hereby declare as follows:

1. I am a Principal Scientist at Monogram Biosciences, Inc. I received my bachelor's degree in Organic Chemistry from the University of Constantine, Algeria. I completed my doctoral training in Organic Chemistry at Strathclyde University, Glasgow, Scotland. In addition, I was a Postdoctoral Research Associate at the State University of New York at Stony Brook, and a Staff Scientist at the University of California, Berkeley. A true and correct copy of my *Curriculum Vitae* is attached to this declaration as Exhibit A. If called as a witness I could competently testify to the facts and opinions expressed in this declaration.

2. My primary research at Monogram Biosciences relates to assay development, and design and synthesis of cleavable eTags for bio-conjugation to proteins and antibodies and for gene

expression products. I have also supervised and managed the Oncology Reagent Group. The research I have conducted has been reported in peer-reviewed publications. In addition, I am an inventor of nine patents and patent applications.

3. For the last nine years I have developed designed, synthesized, and evaluated cleavable tags and applied these compounds to gene expression products and quantification of biomarkers in oncology research. I am the author of several peer-reviewed publications, listed in my *Curriculum Vitae*, which relate to the subject matter described and claimed in pending U.S. patent application 10/740,079 ("the '079 application"). I understand that the Examiner has rejected the pending claims under 35 U.S.C. section 112, first paragraph, as failing to comply with written description. I further understand that the Examiner has rejected the pending claims of the '079 application because the claims allegedly are not enabled under 35 U.S.C. section 112. For reasons I describe below, it is my opinion, that a person of ordinary skill working at the time the invention of the '079 application was made would recognize what has been invented, and practice the claimed invention without undue experimentation.

4. As an author of the above referenced papers, I am intimately familiar with the multiplexed assays described and claimed in the '079 application for "monitoring the level of transcription of one or more genes in response to one or more potential regulatory stimuli."

5. Multiplexed assays described and claimed in the '079 application are used for simultaneous quantitation of transcription levels from multiple promoters, multiple drugs, or for monitoring effects on multiple protein-protein interactions. The methods comprise placing transfected cells in each of a plurality of wells, where the cells in each well are transfected with a genetic construct comprising a selected promoter operatively linked to the coding sequence for an enzyme having a selected enzymatic activity. Then, a probe selected from a set of probes is

added to the cells in each well, where each probe in the set is cleavable by the enzyme into a substrate moiety and an electrophoretic tag (e-tag) reporter having a detection group and a separation modifier that confers on the e-tag reporter, a unique electrophoretic mobility with respect to the e-tag reporters derived from the other probes in the set. The cells and associated probes are incubated the while exposing the cells to a potential regulatory stimulus, obtaining the tags from the cells are obtained and electrophoretically separated, and from the electrophoretic mobility and level of detection group of each separated e-tag reporter, the level of transcriptional response of each cell to the potential regulatory stimulus to which the cells were exposed is obtained.

6. As defined in 2163 et seq. of the Manual for Patent Examining Procedure, the written description requirement is satisfied when the description clearly allows persons skilled in the art to recognized what has been invented. *In Re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ 2d 1614, 1618 (Fed Cir. 1989).

7. It is my opinion that the multiplexed assays outlined and claimed in the '079 application satisfy the requirements for written description because the specification clearly allows persons skilled in the art to recognized what has been invented. The structural information, for example sequence information of an enzyme having selected enzymatic activity, would be readily available to one skilled in the art once the enzyme has been selected. For example, the enzyme selected could be β-lactamase, β-galactosidase, an esterase, a protease, or a nuclease. The sequence information for these enzymes was readily available at the time the invention was made, and the enzymes were readily available from commercial sources.

8. It is my opinion that the specification provides structural information on the separation modifier for the probes used in the '079 application. For example, Figures 1A-1C illustrate a

probe 10 and its transformations through the course of the method of the invention. The probe generally includes a substrate 20 that is linked to a tag that includes a detection group 21, such as a fluorescent reporter, and a separation modifier M_j, indicated at 22, that imparts to the tag a selected separation characteristic, such as, e.g., charge-to-mass ratio, and thus a selected electrophoretic mobility. Further, Figures 10A and 10B show electropherograms corresponding to an assay result before exposing the cells to stimulus (10A), and after exposure to a stimulus that induces transcription of the reporter gene under the control of promoter P_1, but not the genes under the control of promoters P_2 and P_3 (10B). Thus, based on the specification of the '079 application and what was known in the art at the time the invention was made, one of skill in the art could readily determine which separation modifiers to use.

9. As defined in 2164 of the Manual for Patent Examining Procedure, the enablement requirement is satisfied when the specification describes how to make and how to use the invention defined by the claims of the patent application or patent.

10. It is my opinion that methods outlined and claimed in the '079 application satisfy the requirements for written description because the specification clearly allows persons skilled in the art how to make and how to use the invention.

11. The specification of the '079 application provides detailed teaching on the design of probes that can be cleaved by β-lactamase and nuclease, and Figures 10A and 10B show electropherograms corresponding to an assay result before exposing the cells to stimulus (10A), and after exposure to a stimulus that induces transcription of the reporter gene under the control of promoter P_1, but not the genes under the control of promoters P_2 and P_3 (10B).

12. Based on my experience in assay development research and the literature available at the time the '079 application's invention was made, it is my considered opinion that a person

having ordinary skill in the art, working at the time the invention was made, would have readily determined the level of transcriptional response of each cell to the potential regulatory stimulus to which the cells were exposed from the electrophoretic mobility and level of detection group of each separated e-tag reporter.

13. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 3-10-2008 By: A. Chenna

Ahmed Chenna, Ph.D.

Exhibit A

AHMED CHENNA, PH.D.

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HIGHLIGHTS OF QUALIFICATIONS

- Ph.D. in Organic Chemistry
- Co-inventor of cTag™ technology for gene expression and proteomics
- Played a key role in the development and validation of cTag™ Gene Multiplex & Proteomics Multiplex products
- Strong background in the chemical synthesis, bio-conjugation, separation, characterization & analytical methods.
- Experience in assay development and improvement.
- Strong record of scientific productivity as evidenced in over 30 publications & patents
- Creative, motivated, good team-player, effective communicator & flexible

PROFESSIONAL EXPERIENCE

Principal Scientist/ Lab. Manager Aclara Biosciences Inc., / Monogram Biosciences Inc., **1999 to present**

- Conjugation & purification of different antibodies used for the quantification of biomarkers in oncology research
- Conjugation & purification of oligonucleotides with different c-Tags for gene expression product.
- Design & synthesis of cleavable cTags used for the bio-conjugation of proteins & antibodies
- Design, syntheses & characterization of unnatural amino acids used for peptides synthesis
- Design, synthesis and evaluation of various novel molecules used for the conjugation of nucleic acids
- Synthesis and characterization of derivatives of fluorescent dyes with different linkers (PEG & others) and charges used for both oligonucleotides & peptides conjugation.
- Worked on thee assay development and improvement for the quantification of the biomarkers in cancer
- Multi-steps synthesis of new modified nucleosides and phosphoramidites used for the synthesis of DNA
- Developed multi-steps syntheses for large-scale phosphoramidites of various compounds for DNA synthesis
- Experienced in all characterization techniques for large molecules such as MS, HPLC, FPLC, UV and CE.

Supervised & Managed the Oncology Reagent Group.

- Provides the oncology research & development groups with all reagents needed for the development of the oncology products, such as conjugated antibodies, proteins & peptides with different c-Tags (Dyes).
- Purification of antibodies with HPLC, FPLC, characterization & QC.

Supervised & Managed Oligonucleotides Lab.,

- Manufacture of all custom synthetic DNA primers and labeled probes with cTags used for the gene expression.
- Quality control of probes containing cTags with Capillary Gel Electrophoresis
- Maintained four ABI 394 and one ABI 3900 DNA synthesizers
- Maintained five HP 1100 series HPLC's used for DNA analysis and purification

Supervised & Managed Peptides Lab.,

- Synthesis of peptides with and without cTags
- Purification and analysis of peptides and labeled peptides with cTags by HPLC

Staff Scientist, University of California, Berkeley **1993-1999**

- Co-principal investigator with Dr. B. Singer of two NIH-sponsored research Grant proposals, titled "Chemical Basis of Carcinogenic Risk from Benzene" and "Biochemical Mechanism of Exocyclic Adducts in Cancer"
- Design and synthesis fully protected phosphoramidites of novel modified nucleotides and their incorporation into DNA oligomers for various research applications such as DNA repair, site-specific mutagenesis in vitro and in vivo, and for physical studies (1H NMR, Tm)
- Experience in DNA replication: DNA 32P-Labeling and site-specific mutagenesis studies in vitro by using DNA polymerases enzymes
- Knowledgeable about DNA enzymes repair and molecular modeling

Postdoctoral Research Associate, State University of New York at Stony Brook **1990-1993**

- Investigated and identified a series of DNA adducts formed from the reaction of mutagenics and carcinogenic compounds with nucleosides, nucleotides and DNA by using HPLC, I.C, LC/MS and GC/MS (SIM) after derivatization

Graduate Student, Strathclyde University, Glasgow, Scotland, (UK) **1986-1990**

- Designed and synthesized a series of a novel compounds tested for their potential biological activity in Central Nervous System (CNS) by Organon Laboratories Ltd., UK
- Investigated the approaches to the syntheses of Podophyllotoxin and Steganecin and analogues as potential anticancer agents

EDUCATION

- Ph.D. in Organic Chemistry, Strathclyde University, Glasgow, Scotland, (UK) **1986-1990**
- B.Sc. Organic Chemistry, University of Constantine, Algeria **1981-1985**
- Leadership & Management Training Course **2001&2005**

MANAGEMENT AND LEADERSHIP

- Managed & supervised a group of six research associates in Aclara Biosciences & Monogram Biosciences Inc.
- Supervised postdoctoral fellows, graduate students and research assistants
- Environmental Safety and Health Coordinator for the whole Laboratory
- Budget Administrator for group wide purchasing

AWARDS & HONORS

- Co-principal investigator of two NIH-sponsored research grant proposals
- Government Scholarship, Ministry of Higher Education, Algeria, (1986-1990)
- Dean's Honor List, University of Constantine, Algeria (1982-1985)

PUBLICATIONS & PATENTS Over 30 publications, conference articles and patents.

REFERENCES Available upon request

PUBLICATIONS

1: Prediction of Responsiveness to Trastuzumab Treatment in Breast Cancer Patients by EGFR/HER Receptor Dimer Measurement Toi, Saji, Ali Mukherjee, Mengxiang Tang, Liching Cao, Yuping Tan, Rajeev Dua, Yining Shi, Jagrup Pannu, **Ahmed Chenna**, Hasan Tahir, Sharat Singh (Submitted to J. Clin. Oncology).

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4: **Chenna A**. Perry A, and Singer B. Synthesis of 8-(Hydroxymethyl)-3,N4-etheno-2'-deoxycytidine, a Potential Carcinogenic Glycidaldehyde Adduct, and Its Site-Specific Incorporation into DNA Oligonucleotides. **Chem Res Toxicol**. 2000, 13 (3): 208-13.

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6: Maruenda H, **Chenna A**, Liem K L. and Singer B. Synthesis of 1,N6-ethano-2'-deoxyadenosine, a metabolic product of 1,3-bis(2-chloroethyl)nitrosourea (BCNU) and its incorporation into oligmeric DNA, **J. Org. Chem**. 1998;(63),4385-9.

7: Hang B, **Chenna A**, Sagi J, Singer B. Differential cleavage of oligonucleotides containing the benzene-derived adduct, 1,N6-benzetheno-dA, by the major human AP endonuclease HAP1 and Escherichia coli exonuclease III and endonuclease IV. **Carcinogenesis**. 1998 Aug; 19(8): 1339-43.

8: Sagi J, **Chenna A**, Hang B, Singer B. A single cyclic p-benzoquinone adduct can destabilize a DNA oligonucleotide duplex. **Chem Res Toxicol**. 1998 Apr; 11(4): 329-34.

9: Litinski V, **Chenna A**, Sagi J, Singer B. Sequence context is an important determinant in the mutagenic potential of 1,N6-ethenodeoxyadenosine (epsilonA): formation of epsilonA basepairs and elongation in defined templates. **Carcinogenesis**. 1997 Aug; 18 (8): 1609-15.

10: **Chenna A**, Singer B. Synthesis of a benzene metabolite adduct, 3"-hydroxy-1,N2-benzetheno-2'-deoxyguanosine, and its site-specific incorporation into DNA oligonucleotides. **Chem Res Toxicol**. 1997 Feb; 10 (2): 165-71.

11: Hang B, **Chenna A**, Fraenkel-Conrat H, Singer B. An unusual mechanism for the major human apurinic/apyrimidinic (AP) endonuclease involving 5' cleavage of DNA containing a benzene-derived exocyclic adduct in the absence of an AP site. **Proc Natl Acad Sci USA**. 1996 Nov 26; 93 (24):13737-41.

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13: Hang B, **Chenna A**, Rao S, Singer B. 1,N6-ethenoadenine and 3,N4-ethenocytosine are excised by separate human DNA glycosylases. **Carcinogenesis**. 1996 Jan; 17(1): 155-7.

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2. Dua, R., Shi Y., Cao, L., Tan, Y., Murkherjee, A., Pidaparthi, S., Jarvis, L., Pannu, H., **Chenna, A**., Tahir, H., and Singh, S. ErbB/HER Pathway Profiling in formalin-fixed Paraffin Embedded Tissues Using Multiplexed Proximity-based Assays ASCO May 2005 and AACR April 2005

3. Murkherjee, A., Pannu, H., Cao, L., Gangakhedkar, S., Tan, Y., Dua, R., Tahir, H., **Chenna, A**., Mengxiang, T., Singh, S. The use of ErbB/HER Activation Status as Prognostic Markers in Breast Cancer Patients Treated with Herceptin ASCO May 2005 and AACR April 2005

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